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TECHNICAL NOTE

Microsatellite repeat motif and amplicon length affect amplification success of degraded faecal DNA

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Abstract Degradation reduces DNA quality and quantity in faecal samples and leads to low amplification success. We investigated the influence of repeat motif and amplicon length by comparing the amplification success of five dinucleotide, five tetranucleotide, and two compound microsatellite markers for African elephant (*Loxodonta africana*) faecal DNA samples. We found that both repeat motif and amplicon length influenced amplification success, and suggest the use of simple microsatellite markers containing alleles with small amplicon sizes to maximise amplification success of degraded DNA.

Keywords Repeat motif · Amplicon length · Degraded DNA · Faecal DNA · African elephant · *Loxodonta africana*

Introduction

Faecal samples often contain low quality and quantity of DNA (Murphy et al. 2007) and therefore require the use of methods that may increase amplification success (e.g. Piggot et al. 2004; Lampa et al. 2008). Marker repeat motif and amplicon length have inconsistent effects on amplification success (Broquet et al. 2007; Ishida et al. 2012). It may be necessary to choose markers with the highest likelihood of amplification success. The influence of repeat motif and amplicon length on amplification success may dictate marker choice. Our case study on faecal samples from free-ranging African elephants (*Loxodonta africana*) (categorised as vulnerable according to the IUCN redlist) therefore investigates the influence of marker repeat motif and amplicon length on the amplification success of five dinucleotide, five tetranucleotide, and two compound microsatellite markers.

Methods

Laboratory analysis

One-hundred African elephant faecal samples, collected (following Vidya and Sukumar 2005) in the Kavango–Zambezi region (southern Africa), were genotyped for 12 microsatellite markers (Table 1 Online Resources). Our DNA extraction protocol followed the Qiagen (Southern Cross Biotechnology, Cape Town, SA) DNA stool mini kit protocol for human stool, but included increased vortex, centrifuge and incubation times. We used the Qiagen Multiplex PCR kit and Applied Biosystems 3,130 xl and 3,500 xl automated sequencers (Life Technologies, Carlsbad, US) for fragment analysis, and GeneMapper® Software version 3.7 (Applied Biosystems, Foster City, USA) to view and score data. The genotyping process (PCR and fragment analysis) was repeated up to four times, after which unclear genotypes were scored as missing data. We calculated amplification success as the number of

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individuals for which genotypes could not be determined divided by the total number of individuals that where genotyped per locus (n = 100), where amplification success decreases as missing data increases.

Statistical analysis

We used linear regression analysis to assess amplification success as a function of amplicon length (in base pairs of the largest allele size). We used unpaired two-tailed *t* tests to determine if dinucleotide and tetranucleotide amplicon length differed significantly, and to compare the amplification success of simple[1] [dinucleotide markers (n = 5)] and complex markers [tetranucleotide (n = 5) and compound markers (n = 2)]. Statistical analyses were done using GraphPad Prism version 3.00 (GraphPad Software, San Diego California, USA). We identified genotype errors by comparing observed and expected allele frequencies and calculated significance values using Fisher's probability test in the program MICROCHECKER© version 2.2.3 (Van Oosterhout et al. 2004). We excluded these genotype errors in the amplification success calculations.

Results

Missing data increased with amplicon length (Slope = 0.059 ± 0.019; $F_{10} = 9.33$; $p < 0.05$) and nearly half ($r^2 = 0.48$) of the variability in missing data could be explained by differences in the marker amplicon length (Fig. 1). There was no significant difference ($t = 1.56$; $df = 8$; $p > 0.5$) between the length of dinucleotide (mean = 171.4; SD = 59.94) and tetranucleotide (mean = 232.6; SD = 64.47) amplicons (Fig. 1 Online Resources). Simple microsatellite markers (mean = 2.6, SD = 1.52) had significantly fewer missing data ($t = 3.06$; $df = 10$; $p < 0.05$) than complex markers (mean = 11.3; SD = 6.13) (Fig. 2). Five markers showed excessive homozygotes (Table 2 Online Resources), but no locus showed evidence of scoring error due to stuttering or large allele dropout. Two loci (Lat18 and FH19—Table 2 Online Resources) had excessive homozygotes at more than one size class.

Discussion

Amplification success decreased with increasing amplicon length, thus supporting earlier findings of Ishida et al. (2012) who redesigned microsatellite primers to produce shorter amplicons that were more effective in amplifying faecal DNA than the original primers were. In our study repeat motif also influenced amplification success and simple markers had significantly fewer missing data than complex markers. Complex markers may have more difficulty amplifying short fragmented strands of degraded DNA. The similarity in amplicon lengths of dinucleotide and tetranucleotide markers suggests that complexity, rather than length alone, influences amplification success.

We do not know if the excessive homozygotes present in five of our markers occurred naturally in the population or whether they were due to genotype errors. The latter may require the incorporation of allele adjustments provided in MICROCHECKER© and/or the elimination of markers Lat18 and FH19 for future research.

We recognise that repeat motif and amplicon length are only two of a multitude of factors that influence amplification

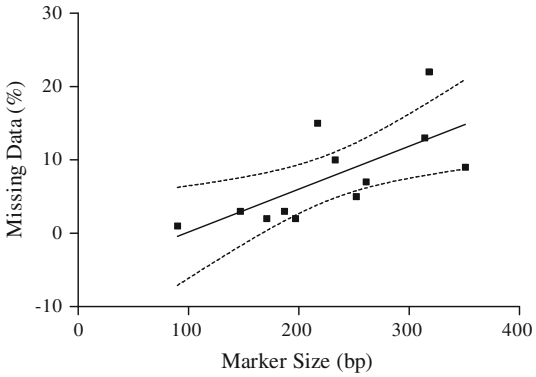

Fig. 1 Missing data per locus is significantly positively related (Slope = 0.59 ± 0.019; $F_{10} = 9.33$; $p < 0.05$; $r^2 = 0.48$) to amplicon length. The *regression line* (*least squares line*) is indicated by the *solid black line* with the 95 % CI indicated by the *dotted lines*

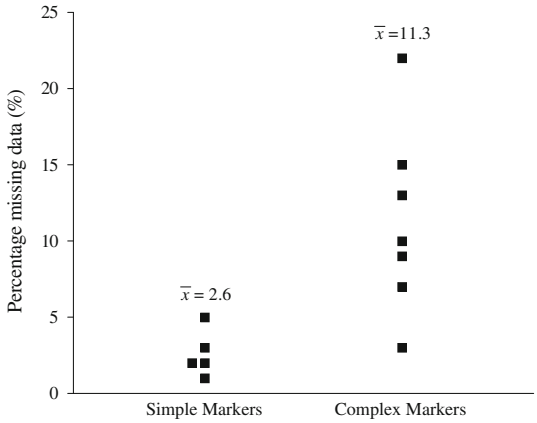

Fig. 2 Simple microsatellite markers (n = 5; mean = 2.60, SD = 1.52) had significantly less missing data ($t = 3.06$; $df = 10$; $p < 0.05$) than complex markers (n = 7; mean = 11.33; SD = 6.13)

[1] In the context of this paper, we use the word "simple" to refer to the less complicated repeat motif of dinucleotide markers as compared to more complex repeat motifs of tetranucleotide or compound microsatellite markers.

success. For example, field conditions (Murphy et al. 2007), preservation methods (Frantzen et al. 1998), and animal diet [e.g. PCR inhibitors in plants (Huber et al. 2002)] may also influence amplification success.

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